In Search of Novel Molecular Targets in Cancer: The Diacyglycerol Pathways

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One of the challenges of cancer therapy is to increase the selectivity of anticancer treatments. Conventional chemotherapies are usually non specific, leading to undesirable toxic effects on normal cells. Therefore, an area of active research in cancer pharmacology is the investigation of the molecular processes critical for tumor growth and metastasis that could be specifically targeted in cancer treatment. In recent years, there have been amazing developments in this area, with the discovery of novel anticancer treatments that target specific cell signaling pathways, such as Herceptin® (Trastuzumab), a monoclonal antibody against the Her-2 tyrosine kinase, and Gleevec® (Imatinib Mesylate), an inhibitor of the BCR-ABL tyrosine kinase. Continuous advances in cancer biology and cell signaling have revealed new molecular pathways that are important in the development and progression of cancer. The diacylglycerol signaling pathways is one of these.

Diacylglycerol signaling molecules: the PKC family

Diacylglycerol is a major lipid second messenger in the cell, produced by the action of phospholipase enzymes. One of the most prominent families of intracellular diacylglycerol targets is protein kinase C (PKC) (Figure 1), a group of serine/threonine kinases that transduces diacylglycerol into a wide variety of signals influencing cell proliferation, differentiation and survival.[1]

The biology of PKC and its role in disease has been extensively studied using the phorbol esters as pharmacological probes. Phorbol esters are a group of natural products that behave as structural mimetics of diacylglycerol in the cell and as such, they bind and activate PKC.[2] The discovery that a subset of phorbol esters are potent tumor promoters has provided the first link between PKC and cancer.[3] Further studies have revealed other functions for PKC, and it is now clear that PKC not only participates in tumor promotion but also in tumor progression and metastasis.[4]

The rationale of PKC as a molecular target in cancer therapy is further supported by the fact that PKC also plays a role in chemoresistance, either by affecting the susceptibility of cancer cells to drugs causing cell death by apoptosis, or by participating in the detoxification of chemotherapeutic drugs by modulating multi-drug transporters.[5] Thus, modulation of PKC isoforms can serve as an adjuvant therapy to make cells more sensitive to the cytotoxic effects of standard chemotherapeutic treatments. In fact, most of the current clinical trials to evaluate PKC modulators are examining these drugs as potential adjuvant agents.

Chemotherapeutic drugs that target PKC

The prototype of the PKC activator with antitumor activity is bryostatin 1, a natural product that, like the phorbol esters, is a structural analog of diacylglycerol (Figure 1).[6] Bryostatin 1 was first discovered as a result of its antileukemic properties *in vitro*.[7] Despite the promising initial activity, bryostatin 1 has shown a very modest effect as a chemotherapeutic drug. Nevertheless, it appears to be useful as an adjuvant by enhancing the effect of various standard chemotherapeutic agents, such as cisplatin, vincristine, and paclitaxel.[8-10]

In addition to bryostatin 1, three other drugs that target PCK have been, or are still being, evaluated in clinical trials for treatment of cancer (for more information go to http://www.clinicaltrials.gov/). These include sanfingol, staurosporine and LY317615. Sanfingol, a lipid derivative that binds PKC, has been shown to potentiate the cytotoxicity induced by chemotherapeutic drugs like ara-C and fenretinide.[11,12] Sanfingol appears to modulate other targets in addition to PKC, however, there is currently no active clinical trial to evaluate this drug in cancer therapy. The staurosporine derivatives UCN-01 and PKC412 are currently being tested in clinical trails as single agents or in combination with standard chemotherapy. Although staurosporine was originally described as a PKC inhibitor, we now know that it can bind other kinases and it is uncertain whether the potential anticancer effects of the staurosporine derivatives are due to modulation of PKC exclusively.[13,14] Finally, LY317615 is a selective inhibitor of PKCα with antiangiogenic activity. This compound is currently being tested in phase I and II clinical trials in the treatment of gliomas and lymphomas.

A novel approach for targeting PKC in cancer therapy is the use of antisense oligonucleotides. ISIS3521 – also known as Affinitak and LY90003 - is an antisense inhibitor of PKCα. ISIS3521 has been evaluated in clinical trails in combination with paclitaxel and cisplatin for the treatment of non-small cell lung carcinoma. Unfortunately, the trial did not show any clinical benefit.[15] It is important to note that PKCα levels were not measured during the trial; therefore, it is still unknown whether the negative results were a consequence of a poor drug or the wrong target selected.

Non-PKC targets of diacylglycerol: chimaerins, Munc13, PKD and RasGRP

For many years, the effects of diacylglycerol, the tumor promoting phorbol esters and the bryostatins were thought to be mediated solely by PKC. We now know that PKC is not the sole target, as four

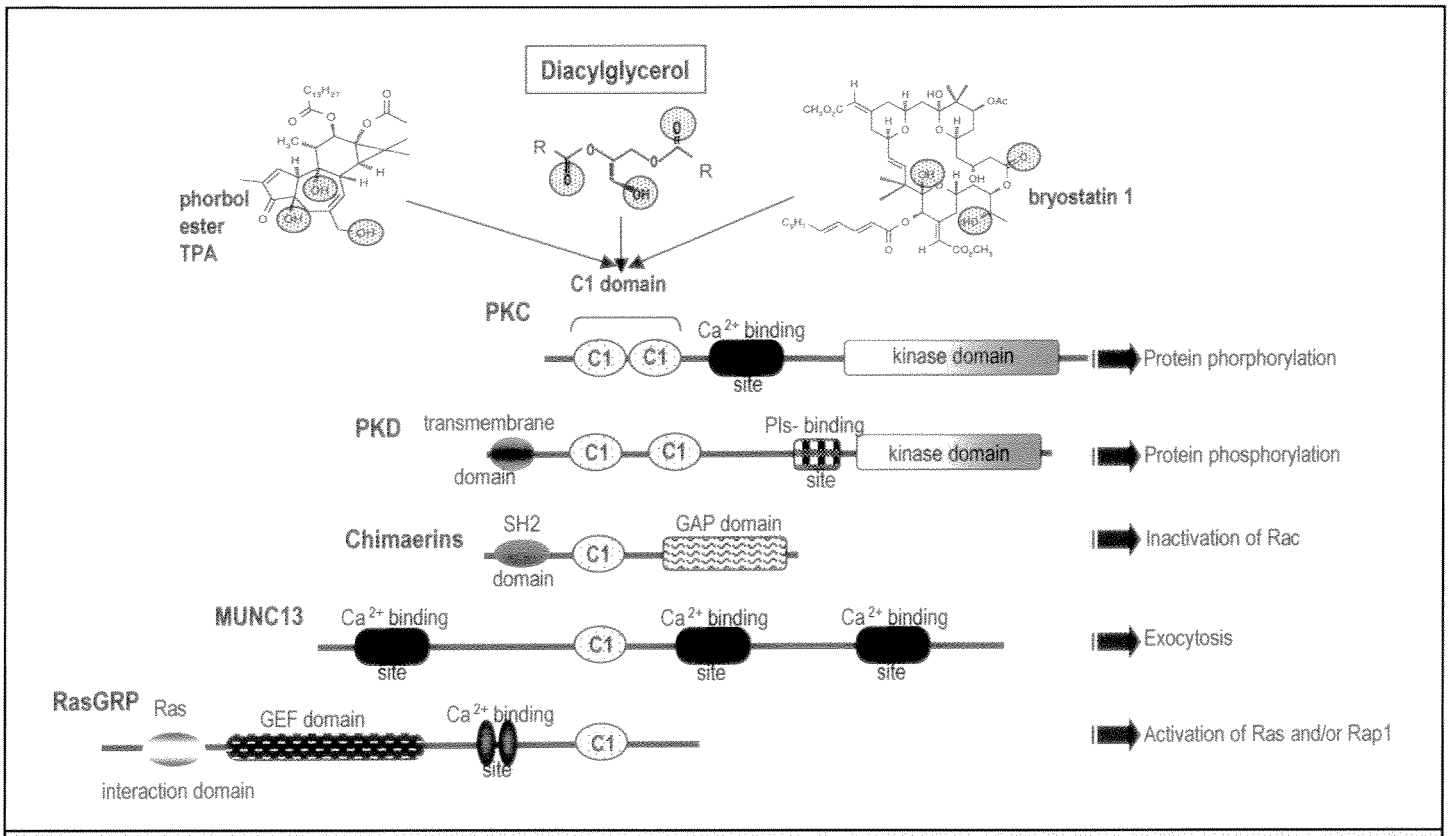

Figure 1.— Diacylglycerol receptors.
The gray circles in the structure of diacylglycerol, phorbol ester and bryostatin 1 highlight the functional groups that are essential for binding to PKC and non-PKC diacylglycerol receptors. C1 represent the domain responsible for binding to diacylglycerol and its structural analogs. TPA: 12-O-tetradecanoyl-13-phorbol acetate; PIs, phosphoinositides; SH2, SRC homology 2 domain (phospho-tyrosine binding domain); GAP, GTPase activator protein domain; GEF, guanyl nucleotide exchange factor domain.

additional families of diacylglycerol targets have been recently discovered: the chimaerins, the Munc13 proteins, the PKD kinases and the RasGRP family (Figure 1).[16] One emerging question is whether these non-PKC receptors could also contribute to carcinogenesis and tumor progression, and therefore, could be potential therapeutic targets in cancer. Our laboratory at the Cancer Research Center of Hawaii is addressing this question for RasGRP.

The RasGRP family is a particularly attractive therapeutic target in cancer because its modulate Ras, a molecule that is implicated in a significant fraction of human malignancies. Our early work has contributed to the characterization of RasGRP as a high affinity target for the tumor promoting phorbol esters, and we recently described that one of the RasGRP members -RasGRP1- functions as a receptor for the phorbol esters in epidermal keratinocytes, which are the target cells for tumor promotion in skin.[17,18] Studies from other laboratories have also linked RasGRP1 with cancer. Recent data has identified RasGRP1 as a potential leukemia oncogene based on the results from retroviral insersional mutagenesis.[19]

Conclusions

The pivotal role of diacylglycerol pathways in cell proliferation, differentiation, and apoptosis, provides the rationale for exploiting diacylglycerol receptors as potential therapeutic targets in cancer. Treatments that modulate PKC, the major intracellular diacylglycerol receptor, have been evaluated in clinical trails for the treatment of several malignancies. Bryostatin 1 is the typical PKC modulator that continues to be evaluated as adjuvant of standard chemotherapeutic drugs. The fact that diacylglycerol, phorbol esters and bryostatin 1 can also modulate non-PKC diacylglycerol targets raises the question as to the role of the non-PKC receptors in cancer. The recently discovered properties of the RasGRP as a phorbol ester receptor, Ras activator, and potential oncogene, make this family an attractive novel target for selective intervention. Further studies in the area of diacylglycerol signaling should provide insights into the role of each diacylglycerol receptor in cancer that may eventually lead to the development of novel targeted therapies with enhanced effectiveness and reduced toxicity.

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